PLASTID GENOME ENGINEERING

Carbon nanotube deliverer

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Engineering the maternally inherited chloroplast genomes can prevent the outcrossing of transgenes and produce vast amounts of foreign proteins by introducing thousands of copies of transgenes into one cell. Despite these advantages, chloroplast genome engineering has been a slowly progressing area, largely due to the technical challenges of routine transplastomic approaches. Now, Seon-Yeong Kwak, from Massachusetts Institute of Technology, and colleagues developed a technology that enables efficient chloroplast transformation in different plant species based on chitosan-complexed single-walled carbon nanotubes (SWNTs).

Non-toxic chitosan-wrapped SWNTs can traverse plant cell walls, the plasma membrane and double lipid bilayers of chloroplasts thanks to their high surface charge. Chitosan can complex with negatively charged plasmid DNA (pDNA) through electrostatic interactions and protect DNA from nuclease degradation. Based on these characteristics, the researchers designed four types of chitosan-complexed SWNT gene carriers to enable gene delivery to chloroplasts using a lipid exchange envelope penetration model. Three types of these carriers were based on non-covalent wrapping of nanotubes by chitosan or modified chitosan, while the fourth type was designed by covalent modification of the nanotube sidewall. Different types exhibited different zeta potential and hydrodynamic radius, with the covalently modified type displaying the highest zeta potential and lowest mean hydrodynamic radius. These chitosan-complexed SWNTs can conjugate to pDNA tightly at mildly acidic pH in cytosol, but release pDNA in chloroplast stroma as pH increases due to weaker electrostatic interaction.

Using pDNAs encoding a yellow fluorescence protein (YFP), the researchers monitored the transient expression of YFP in the chloroplasts of *Arabidopsis thaliana* mesophyll protoplasts. Successful delivery of pDNAs to the chloroplasts was observed when the protoplasts were incubated with the covalently bound conjugates. The researchers further showed efficient gene delivery, mediated by two types of these nanocarriers, into the chloroplasts of mature arugula, watercress, spinach and tobacco plants, without affecting leaf lifespan.

This nanoparticle-mediated chloroplast transgene delivery approach may have broad applications as it is simple, cheap and applicable across different species. Moreover, the SWNTs in the transplastomic plants can be removed through multiple rounds of selection and breeding, suggesting the commercial viability of this technology.

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